THE GENETICS OF ARTEMIA SALINA. VI. SUMMARY OF MUTATIONS[1]

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Artemia salina is of interest to geneticists because some populations are diploid, triploid, tetraploid, or pentaploid (see reviews by Goldschmidt, 1952; Barigozzi, 1957; and Stefani, 1964). Although the cytology of the brine shrimp has been studied for many years, it is only recently that attempts have been made to analyze mutant traits governed by one locus. Cervini (1965) has described the spontaneous recessive autosomal mutation "curly" (*cr*) which causes ventral curling of the abdomen. In our study of gonochoristic diploid populations, we have found seven mutations and many sex mosaics and eye color mosaics. The purpose of this paper is to describe these morphological variations.

Materials and Methods

Genetic techniques, glassware, and feeding schedule were described in detail earlier (Bowen, 1962). In brief, two or three nauplii were placed in each vial of culture medium (50 g. NaCl per liter of sea water). Once a week, 0.05 or 0.10 cc. of yeast suspension (1 cc. dry brewers' yeast mixed with 9 cc. of medium) was added to each vial. Inbred stocks are maintained at 21–24° C.; shrimps reach sexual maturity at two to three weeks of age. Origins of the inbred stocks and of the wild populations have been given earlier (Bowen, 1964, 1965).

Macrophotographs of living *Artemia* were taken with a Brinkmann camera (30″ bellows) and collimated transmitted light. For histological preparations, shrimps were anesthetized in ether and a few legs were removed to allow entry of fixative; they were placed in Bouin's for 24–48 hours, stored in 70% ethanol, embedded in paraffin, sectioned at 10 μ and stained in haematoxylin and eosin.

India ink was injected into the thorax through micropipettes with tips of 2–4 μ (O.D.) by means of a de Fonbrune micromanipulator. Best results were obtained when shrimps were first anesthetized with ether and then placed within an enclosure improvised from a plastic slide (Dowling, 1963). At the time of injection, the culture medium was drawn off to prevent loss of ink from the micropipette. *Artemia* is able to survive for a few minutes outside the liquid environment.

Morphology of Wild-Type Artemia

Fränsemeier (1939), Weisz (1947) and Dutrieu (1960) have described embryonic development. Heath (1924) and Weisz (1946, 1947) have outlined the

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changes during larval development. We have used Heath's diagrams to determine the instar of immature shrimps. The morphology of the adult has been described by Weisz (1947) and Lochhead (1950). The adult body consists of head, 11 thoracic segments, two genital segments, and 6 abdominal segments (Weisz, 1947, p. 81). Each of the 11 thoracic somites bears a pair of phyllopodia (Figs. 2 and 5). The last abdominal somite is fused to the telson which bears the caudal furca (Fig. 5).

The head of *Artemia* bears a pair of slender antennules and a larger pair of antennae. The antennae show sexual dimorphism, being larger and modified for clasping in the male (Figs. 1, 2 and 5).

The median eye consists of three cups, or ocelli. It is red in the first instar and does not gain black pigment until the second (Vaissière, 1961, p. 29). By the third instar, black pigment is usually present in the rudiments of the lateral compound eyes also. The normal compound eye is seen in Figures 7 and 12. The cuticle, which is secreted by the epidermal cells, is not thickened to form a lens. Each ommatidium consists of a cone surrounded by the four crystalline cells which

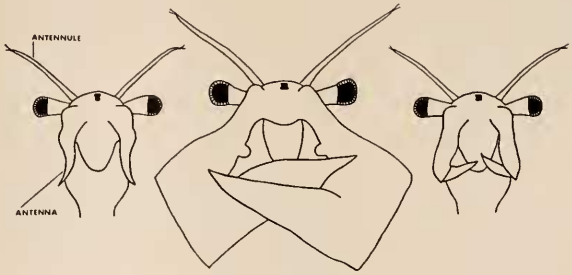
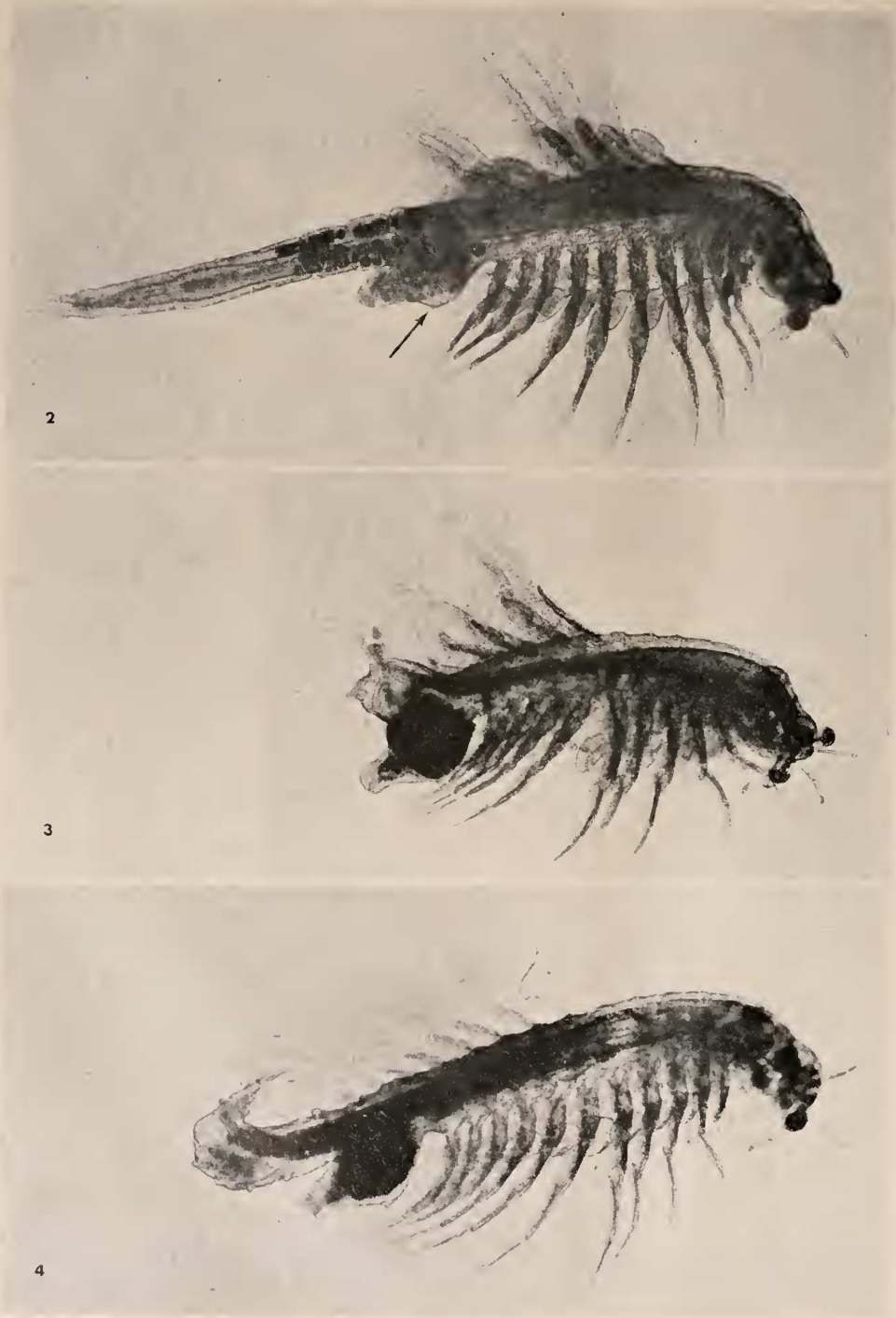


FIGURE 1. Ventral view of head of normal female (left), normal male (center) and *Cu*/+ female with curved antennae (right).

secreted it and a proximal rhabdome surrounded by a retinula. The rhabdome lacks the alternating layers of microtubules found in other crustaceans (Eguchi and Waterman, 1965). Each retinula usually contains 5 principal cells and a sixth accessory cell (Debaisieux, 1944, p. 13). The retinular cells contain the photostable black-brown pigment which gives the wild-type eye its black color. Each retinular cell is a primary neuron which penetrates the basement membrane and continues as an axon in the fascicular zone of the eyestalk. There are two optic ganglia: the distal lamina ganglionaris and a proximal medulla (Fig. 7). Nerves from the ganglia enter the supra-esophageal ganglion.

The gonads of both sexes are straight cylinders lying above and lateral to the gut in the two genital segments and first few abdominal segments. Gametes leave the anterior ends of the gonads by means of ducts. On each side of the body, the male has a U-shaped seminal vesicle, vas deferens, and penis. The female has two oviducts (lateral pouches) which convey the eggs into a single median uterus wherein they undergo segmentation. Four grape-like clusters of shell glands empty their secretions into the uterus. The oviducts and uterus lie within a ventral median swelling, the ovisac (Fig. 2).



FIGURES 2–4.

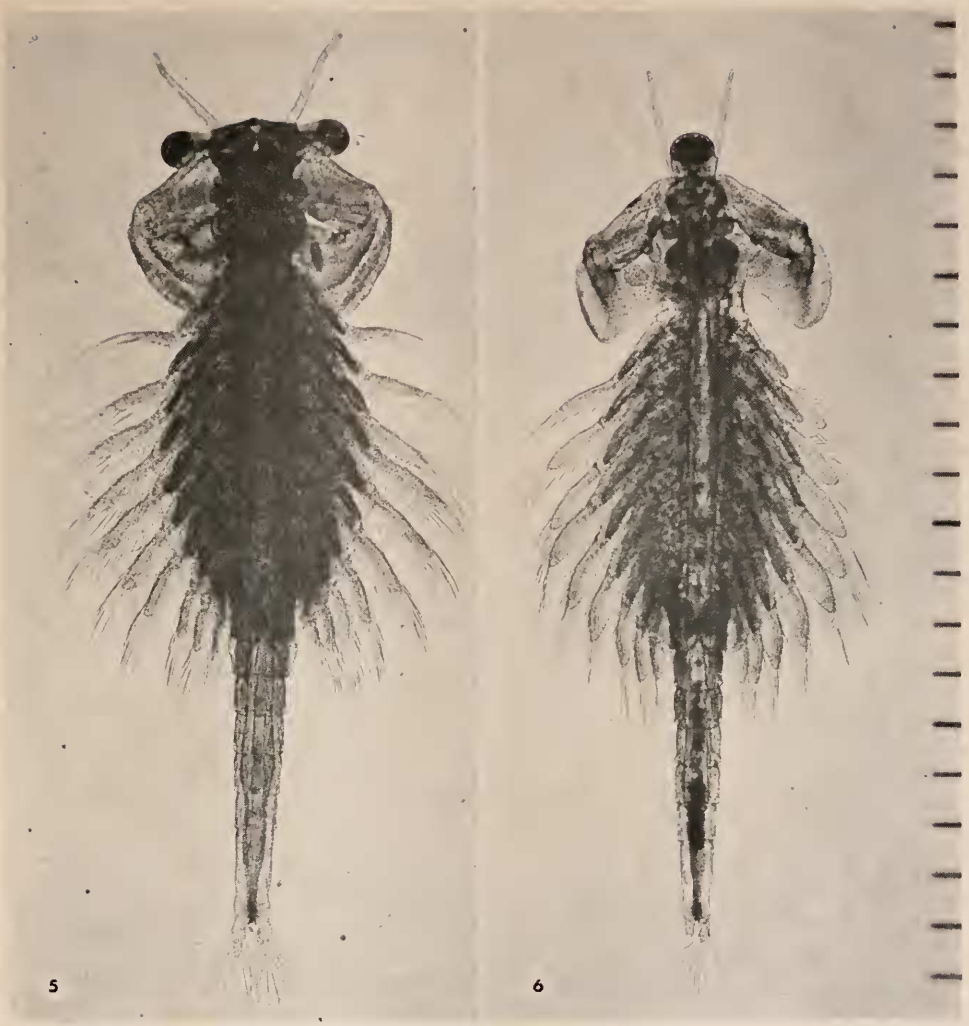


FIGURE 5. Dorsal view of living normal male *Artemia*.

FIGURE 6. Dorsal view of cyclops male. The distance between the lines on the right is 0.5 mm.

MORPHOLOGICAL VARIATIONS

A. *Variations in morphology of wild populations*

Wild-type *Artemia* look very much alike. This is surprising when one considers their geographical isolation: they are found in salt lakes and salterns on

FIGURE 2. Lateral view of living normal female brine shrimp, showing two genital and 6 abdominal segments. The arrow indicates the spine on the ovisac.

FIGURE 3. Lateral view of *s/s* female which has extreme stump expression. Only two genital segments are present.

FIGURE 4. Lateral view of living *s/s* female with moderate stump phenotype.

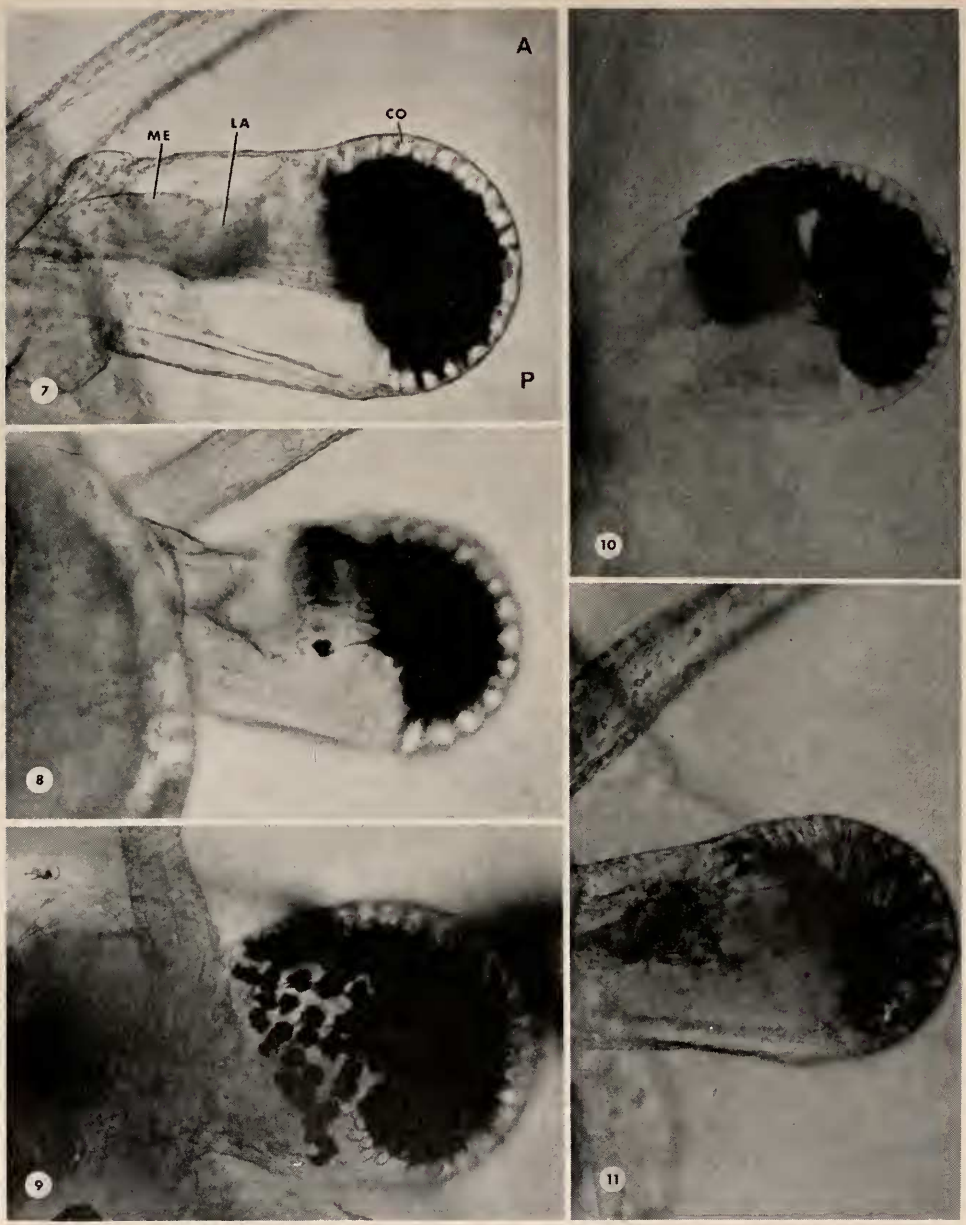


FIGURE 7. Dorsal view of normal compound eye of living brine shrimp. The medulla (ME), lamina ganglionaris (LA) and the cones of the ommatidia (CO) are clearly seen. A, anterior border; P, posterior border of eye. The other photographs on this page are oriented in a similar manner.

FIGURES 8, 9, AND 10. Dorsal views of living shrimps of *c/c* genotype, showing variation of expression of crinkle phenotype.

FIGURE 11. Dorsal view of eye of living garnet shrimp (*g/g* genotype). In areas where the retinular cells have degenerated, the eye is transparent. Few axons remain in the fascicular zone (between lamina and basement membrane).

six continents. Furthermore, certain populations are known to be reproductively isolated. Whereas most American populations are gonochoristic, many European populations are parthenogenetic. American diploid gonochoristic populations are also reproductively isolated from the diploid gonochoristic population from San Bartolomeo near Cagliari, Sardinia (Kuenen, 1939, p. 387; Bowen, 1965). The gonochoristic Mono Lake, California, population is a sibling species which cannot survive in sea water or concentrated brines in which all the other populations thrive (Bowen, 1964).

When reared under identical environmental conditions, some wild-type populations can be distinguished by quantitative differences such as ratio of lengths of abdomen and trunk (see data and review by Gilchrist, 1960). We have examined two parthenogenetic populations (from Sète, France, and from Rottnest Island, near Perth, Australia) and six gonochoristic populations (from Europe, North America and South America) and have detected only a few differences of a qualitative nature. For example, females of the Quemado, New Mexico, population have a small projection on their antennae which is absent in other females (Bowen, 1964). Both males and females from the San Bartolomeo population lack the spikes on the genital segments (seen in Figures 2 and 3) which are present in other populations (Bowen, 1965).

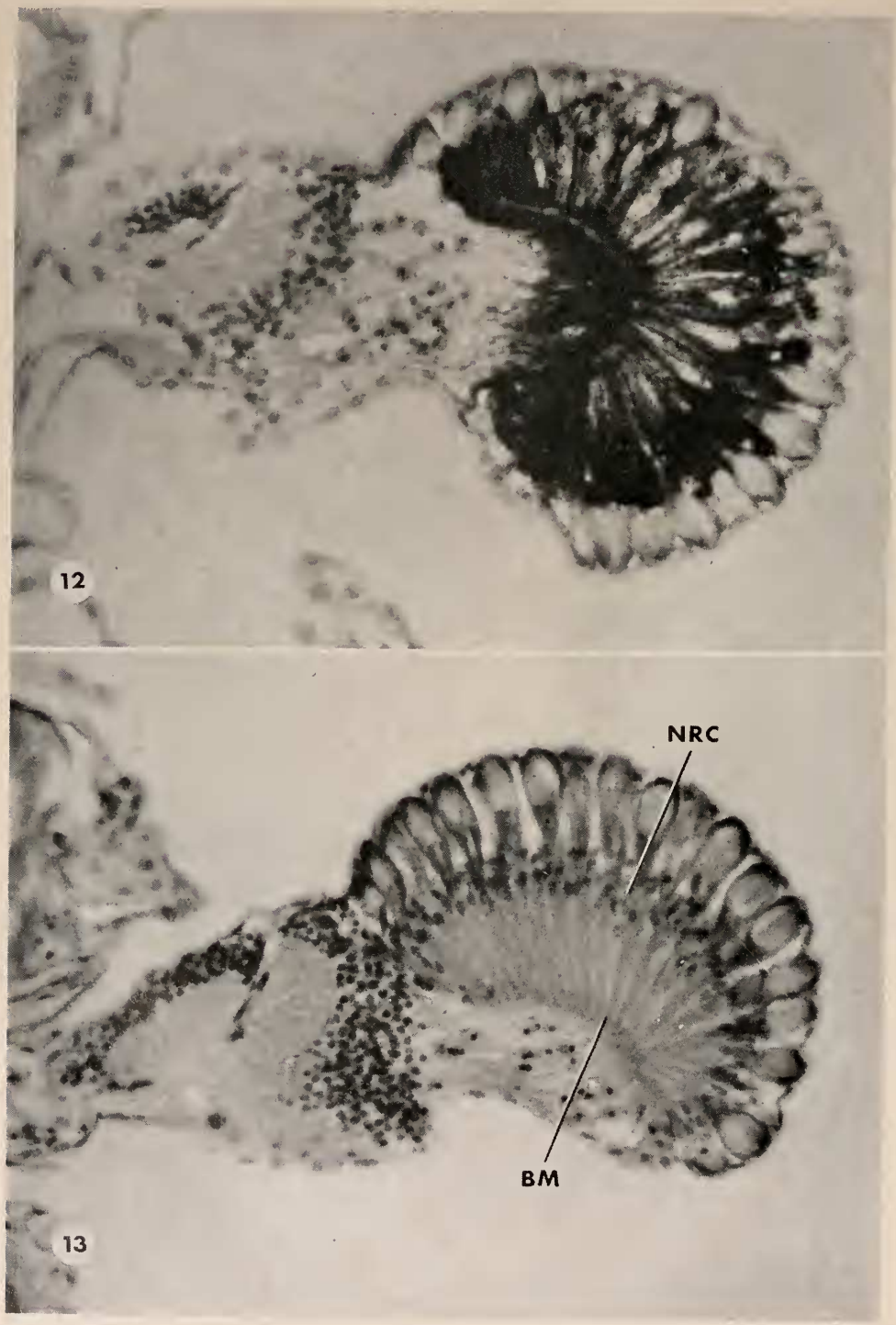
B. *Description of seven mutant genes*

Six of the seven mutations listed below appeared when non-irradiated stocks were inbred by sibling matings; one (garnet) appeared in progeny of x-irradiated shrimps. Two mutations (white and curved) were found by S. T. B.; the other five were discovered by J. H. Five are completely recessive; two (crinkle and curved) have expression in a fraction of the heterozygotes. Six of the seven mutations are carried in our laboratory in pure-breeding cultures; the exception is cyclops which occurs in high frequency in stock #1. Five are autosomal, one (white) is partially sex-linked, and the mode of inheritance of one (cyclops) is not completely known.

1. *Curved* (*Cu*). Males homozygous for the mutant gene are normal; expression is therefore said to be sex-limited. Expression in the females is variable, ranging from enlarged, sharply bent antennae (easily seen without a microscope) to antennae which are normal in size but which have a small projection on the posterior surface (visible only when the female is anesthetized and examined under 30 × magnification). Females with extreme curved expression have antennae similar to those of normal males (Fig. 1).

The first females with curved antennae were discovered in 1965 among the progeny of a cross between inbred stocks #5 and #12. These females were mated to males from an inbred wild-type stock derived from salterns on Pichilingue Island, Mexico. Of the 313 hybrid female progeny, 56 showed strong expression of curved. In retrospect, it seems probable that the first females were *Cu*/+ genotype. The hybrid progeny were inbred for four generations with constant selection of females for strong expression of curved. The result was stock #49 which has high incidence of curved (Table I).

The degree of bending of female antennae increases if animals are reared at



FIGURES 12–13.

27° C. instead of 22° C. At both temperatures, frequency of females with strong expression (detected without use of a compound microscope) increases as the population ages. The *Artemia* described in Table I were reared at 27° C. and classified at an age of five weeks.

Females from the wild-type Pichilingue inbred stock were mated to stock #49 males. Data in the second line of Table I show that 31/52, or 60% of the F_1 females had curved antennae, indicating that this trait is determined by a gene with incomplete dominance which can be transmitted through the male. This excludes the possibility that the curved trait is governed by a gene on the Y chromosome; the female is the heterogametic sex (XY) in *Artemia* (Bowen, 1963a, 1965; Stefani, 1963). The F_1 females were backcrossed to +/+ Pichilingue males. The data in the last line of Table I show that their daughters had curved

TABLE I

Segregation of gene for curved antennae which has expression only in females (classification at 5 weeks of age)

Parental cross		Female progeny classified as curved	
Description	Presumed genotype	Number with strong** expression/total	Number with some*** expression/total
curved stock #49 ♀ ♀ × stock #49 ♂ ♂	*Cu/Cu* × *Cu/Cu*	43/46	46/46
non-curved Pich.* ♀ ♀ × stock #49 ♂ ♂	+/+ × *Cu/Cu*	31/52	41/52
non-curved Pich. ♀ ♀ × ♂ ♂ F_1 (Pich. ♀ ♀ × #49 ♂)	+/+ × *Cu*/+	6/22	8/22
curved ♀ ♀ F_1 (Pich. ♀ ♀ × #49 ♂) × Pich. ♂ ♂	*Cu*/+ × +/+	12/44	16/44

* Pichilingue inbred wild-type stock.

** Strong expression indicates that unanesthetized females were classified as curved after observation under a dissecting microscope (7 ×).

*** Some curved expression includes those with strong expression and those with mild expression (seen only on anesthetized females under 30 ×).

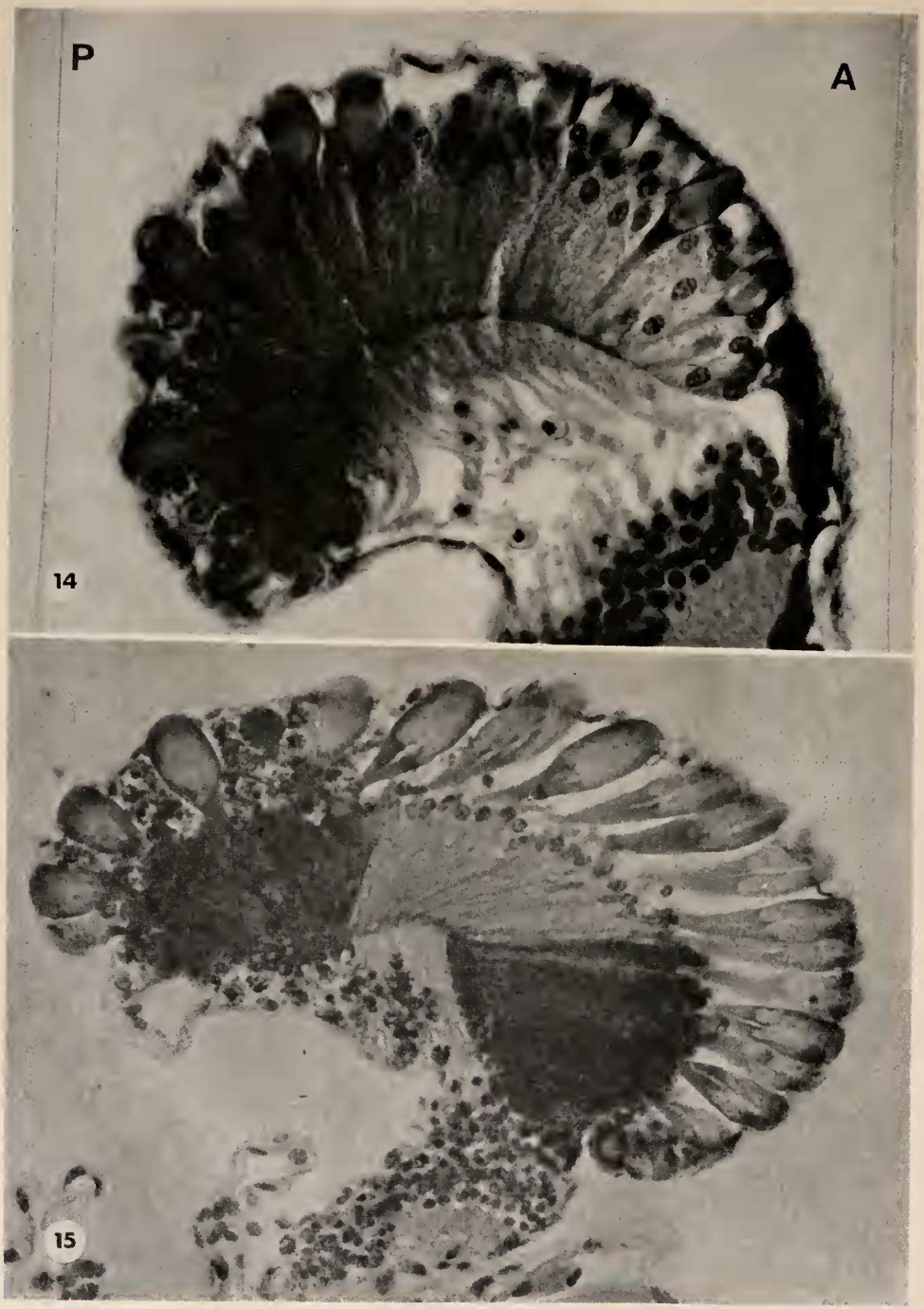
antennae. This demonstrates that curved is not located on the X chromosome. (If curved were on the X, the F_1 females would be $X^{Cu}Y^{+}$ and would be unable to transmit the mutant gene to their female offspring.)

We conclude that curved is a dominant autosomal sex-limited gene with incomplete penetrance and variable expression. Females with mild expression have a projection of the posterior surface of their antennae, as do wild-type Quemado females.

2. *Stump* (*s*). This autosomal recessive mutation was discovered in 1960 during inbreeding of wild-type shrimps from salterns on San Francisco Bay. In some *s/s* shrimps, the abdomen is normal. In others, it is twisted dorsally (Fig. 4), or it lacks from one to six segments. The female shown in Figure 3 lacked all

FIGURE 12. Longitudinal section of normal compound eye. Black pigment is within the retinular cells.

FIGURE 13. Longitudinal section of eye of shrimp with *w/w* (white) genotype. The basal membrane (BM) and the nuclei of the retinular cells (NRC) are seen. The retinular cells contain opaque white pigment. All histological preparations (Figs. 12–17) were prepared in the same manner (haematoxylin and eosin).



FIGURES 14–15.

six abdominal segments. Although her second genital segment was attached directly to the telson, she had normal fertility.

Matings of stump males to stump females gave rise to a stock of *s/s* shrimps in which only 37% of the shrimps showed sufficient expression to be classified as stump (when viewed under a 7 × dissecting microscope). From matings within this *s/s* stock, the ratio of stump to non-stump progeny was the same when extreme stump parents were selected as when non-stump parents were selected. This suggests that the *s* gene has low penetrance in homozygotes.

3. *Red* (*r*). This autosomal recessive mutation appeared during inbreeding of a stock from Great Salt Lake, Utah, in 1960. Segregation data and descriptions of *r/r*, *r/+* and *+/+* shrimps appeared earlier (Bowen, 1962). Briefly, *r/r* shrimps have colorless compound eyes from the third to the fifth instar. Then deposition of red pigment begins in the posterior border and rapidly progresses anteriorly (Fig. 18). Median eye and compound eyes are bright red from the seventh through the thirteenth instars (about two to three weeks of age). Shortly after sexual maturity, brown-black pigment appears in the caudal retinular cells of the compound eyes. Deposition of black pigment also progresses anteriorly, masking the red pigment within 48 hours (Fig. 14). The median eye may also darken, but more often remains red. A similar mutation governs rate of eye pigment production in the amphipod, *Gammarus chevreuxi*. The gene *d* (delayed melanin) delayed deposition of pigment until the amphipods were sexually mature (Ford and Huxley, 1929).

4. *Cyclops* (*cy*). During development of cyclopean metanauplii, the lateral eyes give the illusion of moving forward and fusing together in the midline as a single large compound eye (Figs. 6 and 20). The eyes are in the normal location at the fourth instar; fusion is complete by the ninth instar. Histological preparations indicate that ganglia and nerves of the two optic stalks fuse. The eye of the cyclopean *Artemia* is similar to the normal eye of the cladoceran, *Leptodora*.

Cyclopean *Artemia* occur sporadically in stock #1 (*r/r* genotype) which is descended from the Great Salt Lake population. Nine cyclops were observed in this stock in 1961. Two died before sexual maturity. Of those that matured, four were male and three were female. Only two produced offspring. In the first successful mating, a cyclopean male (*r/r*) was outcrossed to a wild-type female. Of the 27 progeny, only 9 lived to maturity; all were non-cyclopean *r/+* shrimps. These were bred *inter se* but none of the 255 F_2 progeny was cyclopean. In the second successful mating, a cyclopean female was mated to her brother and produced 42 nauplii, of which 11 reached maturity. One was a male cyclops which failed to produce progeny. His sibs were mated *inter se;* of 140 offspring, 75 reached maturity and all had *r/r,* non-cyclopean eyes. This finding is quite different from the results obtained when the sibs of another cyclops were mated: three of the 51 progeny were cyclopean.

FIGURE 14. Longitudinal section through eye of a sexually mature shrimp of *r/r* genotype. A, anterior; P, posterior. Deposition of black pigment has begun in retinular cells in the posterior part of the eye. The anterior portion is still bright red.

FIGURE 15. Section through eye of sex mosaic #14 which was also mosaic for eye color. There is a central patch of white tissue surrounded by pigmented tissue of *r/r* genotype (black pigment in this mature shrimp). Note that pigmented and white retinular cells lie side by side with no areas of intermediate color.

The study of cyclops was abandoned because the cyclopean shrimps had low viability and fertility. The trait may be governed by a recessive gene which has low viability or low penetrance, or it may be affected by more than one locus. A future study might be made of stock #1 in varying ionic environments or at different temperatures, in an attempt to increase the frequency of cyclopean shrimps.

5. *Crinkle* (*c*). In immature shrimps with *c/c* genotype, the compound eyes are normal. After sexual maturity, some retinular cells detach from the ommatidia with the result that the eye becomes mottled in appearance. In *c/c* shrimps six weeks old or older, the distal ends of some retinular cells lie in the eye stalk rather than in the normal eye field. A characteristic "crinkle patch" (containing retinular cells but lacking cones) appears in the anterior dorsal region of the stalk, medial to the basement membrane (Figs. 8, 9 and 10).

The first crinkle-eyed shrimp appeared in 1960 in an inbred stock derived from salterns on San Francisco Bay (Bowen, 1963a). From a backcross of *c/+* to *c/c* shrimps, 116/274, or 42% of the progeny had crinkle phenotype at the age of five weeks. The frequency had increased to 114/224, or 51%, when these backcross progeny reached 7 weeks of age, because expression of *c/c* genotype becomes

TABLE II

Segregation of the gene g

Type of mating	Classification of progeny (at 4 weeks of age)		Total
	Wild	Garnet	
g/g × *g/g*	0	301	301
♂ +/+ × ♀ *g/g*	105	0	105
♂ *g/g* × ♀ +/+	297	0	297
♂ +/*g* × ♀ *g/g*	158	133	291
♂ *g/g* × ♀ +/*g*	261	240	501

more pronounced with age. Unfortunately, the crinkle gene has some expression in a small fraction of heterozygotes and this frequency is also increased with age. Evidence for this is seen in data from a heterozygous F_1 population. At four weeks of age, 3/90 *c/+* shrimps had crinkle eyes; at 10 weeks of age, 8/53 were crinkle-eyed. The frequency of expression in *c/+* heterozygotes may vary with environment and genetic background as well as with age.

6. *Garnet* (*g*). This mutant eye color first appeared in 1961 in the F_2 of two shrimps from San Francisco cysts which had received 10 kr of x-irradiation (Bowen, 1963b). After a pure-breeding stock was established, reciprocal crosses were made between garnet and wild-type and testcrosses were made of the F_1. The data in Table II indicate that garnet eye color is due to an autosomal recessive gene which has complete penetrance in the homozygote and no expression in the heterozygote.

Eye color becomes progressively lighter as the *g/g* shrimp ages. In the first three instars, the eyes cannot be distinguished from wild-type. However, at one week of age (fourth to seventh instar), the eyes become dark brown or red-brown (garnet). This mutation affects both eye color and structure. At sexual maturity

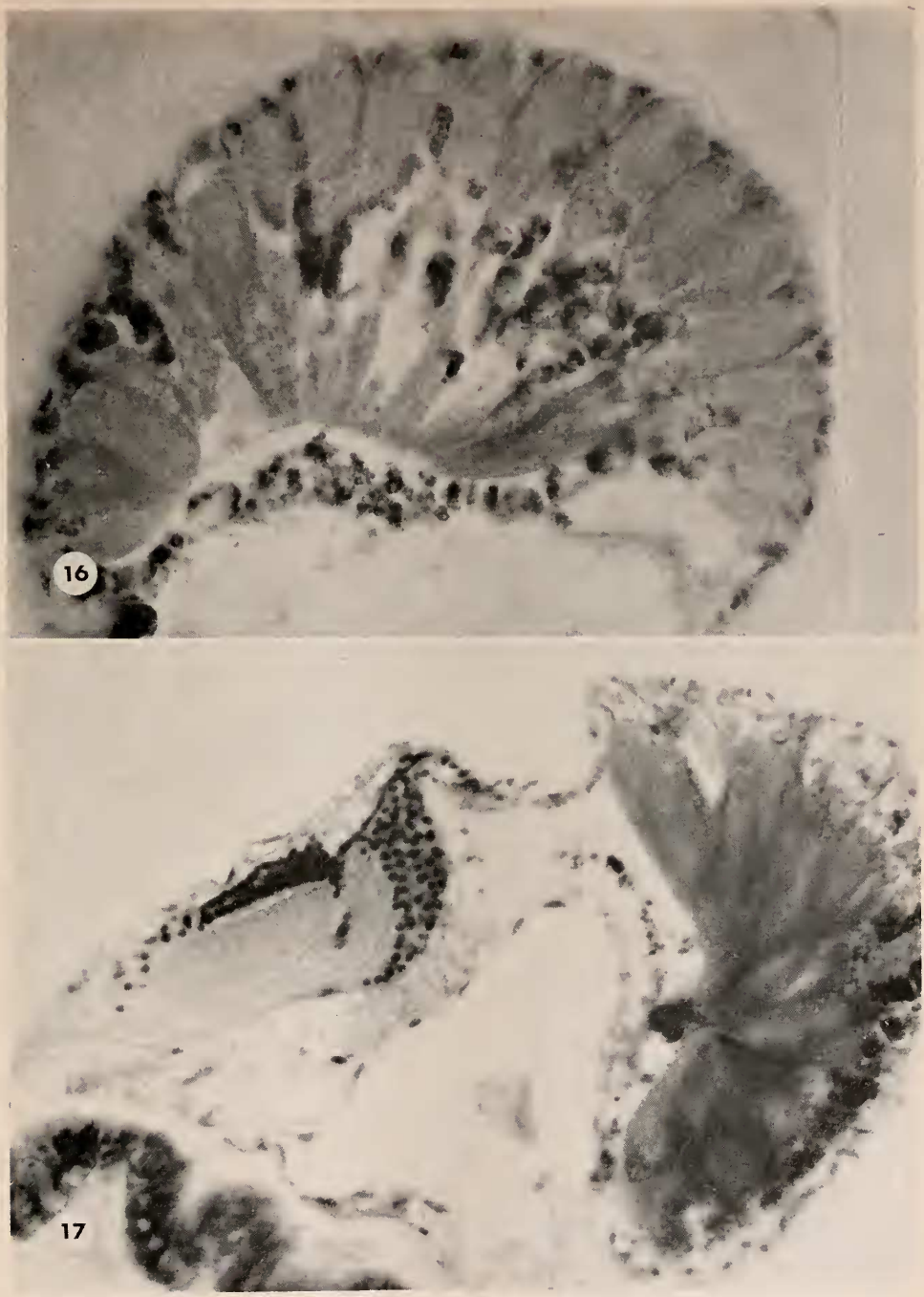


FIGURE 16. Longitudinal section through tip of the eye of a garnet (g/g) shrimp. Whereas the rhabdome remains intact, the retinular cells have degenerated, leaving cell fragments filled with garnet pigment above and below the basement membrane.

FIGURE 17. Longitudinal section through eyestalk of a garnet-eyed shrimp. The retinular cell axons, which normally lie between the basement membrane and the distal optic ganglion, have degenerated.

FIGURES 18–21.

(three weeks of age), the garnet eyes often have an irregular proximal border. Many retinular cells degenerate, causing the eye to be flecked with clear areas. By 6 to 8 weeks of age, the compound eyes of *g/g* shrimps have irregular patches of garnet pigment only at the periphery of the eye; some eyes are almost colorless (Fig. 11). The median eye is often unpigmented also. In histological preparations, it is seen that retinular cells detach from the rhabdome and basement membrane. Many disintegrate. Those that remain are small spherical cells (or cell remnants) which lie medial to the basement membrane or in the periphery of the eye field (Fig. 16). Note that the rhabdome remains structurally intact after degeneration of the retinular cells. At the age of two months, no axons can be seen in the zone between basement membrane and distal ganglion (Fig. 17). By this time, garnet-eyed shrimps have lost the tendency to orient with their ventral surface toward a light source.

Correlated with a decreasing pigmentation of the retinular cells is an increasing deposition of garnet pigment in other specialized cells. By the sixth instar, garnet pigment is seen in the antennal glands. By the eighth instar, pigment is present in the phagocytic storage cells and in the maxillary glands. When *g/g* shrimps reach four weeks of age or more, they have conspicuous pigmented areas around the caudal walls of the lobes of the stomach, on the lateral surface of the anterior part of the digestive tract, and on the outside of the anterior portion of the heart. These are sites where phagocytic storage cells are concentrated. To demonstrate this, wild-type shrimps were injected with India ink. Ink was found in cells in the phyllopodia, in the maxillary glands, and scattered along the outer walls of the heart. These phagocytic cells were particularly dense on the outside of the lateral walls of the gut in the anterior thorax. In Figure 19, the characteristic "inverted U" distribution of ink-filled phagocytic cells across the dorsal surface of the stomach walls is seen. The garnet pigment in mature *g/g* shrimps has an identical distribution.

In order to determine the mechanisms of cellular degeneration and of pigment dissolution, a study should be made of the ultrastructure of the eye in *g/g* shrimps, with particular attention to changes in the lysosomes with age. It is interesting to note that a recessive mutation (albino) which brought about complete degeneration in retinular cells appeared in the amphipod *Gammarus chevreuxi* (reviewed by Sexton and Clark, 1936, p. 365).

7. *White* (*w*). The gene for white eyes is recessive and partially sex-linked; that is, the white locus is on the homologous segment of the sex chromosomes

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FIGURE 18. Ventral view of living sixth instar metanauplius of *r/r* genotype. The median eye is red. The anterior portions of the two compound eyes lack pigment. Red pigment is being laid down in posterior ommatidia; this process will proceed anteriorly until the entire eye becomes red.

FIGURE 19. Dorsal view of head and upper thorax of living wild-type female which has been injected with India ink. Note ink-filled phagocytic cells concentrated in a U-shaped area above the posterior walls of the stomach lobes (SL).

FIGURE 20. Dorsal view of eye of living cyclops male.

FIGURE 21. Lateral view of genital segment of living mosaic male #3 which has one "penis" which is a mixture of ovisac and penis structures proximally and phyllopodium structures distally. The arrow indicates the spine of the "ovisac"; SV, seminal vesicle filled with sperm.

(Bowen, 1963a). In matings of X_wX_w males to X_wY_+ females or to X_+Y_w females, crossing over can be detected. The amount of recombination between the white locus and the sex locus varies from 0.05% to 20%, depending upon which female line is tested. The characteristic crossover frequency is transmitted matroclinously (Bowen, 1965).

The gene *w* has no expression in the heterozygote and has complete penetrance in the homozygote. The lateral and median eyes in *w*/*w* shrimps are white throughout the lifespan with one complication: some *w*/*w* shrimps develop a pink or bright orange cast to their eyes. Orange pigment may be in the retinular cells and/or in the nerve and ganglia in the eye stalk. Attempts to select for orange color in breeding experiments have failed. Further evidence that this trait is not heritable is the fact that if *w*/*w* shrimps with orange eye color are transferred to fresh culture medium, the color will fade within a few weeks, which suggests that the orange tinge must be due to the storage of some material obtained from the food.

If one compares white eyes and garnet eyes (Figs. 13 and 16), one sees that aging garnet eyes become transparent due to degeneration of retinular cells, whereas white eyes contain opaque white pigment in their retinular cells.

C. *Gene interactions* (r, g *and* c) *and linkage*

Shrimps with *c*/*c*; *g*/*g* genotype have reddish-brown eyes as they approach sexual maturity. Three weeks later, crinkle patches on the eye stalk appear but they are difficult to see because at this time the retinular cells degenerate under control of the garnet gene. Shrimps with *c*/*c*; *r*/*r* genotype have dark red eyes as they approach sexual maturity. Three weeks later, the main eye fields turn black, but the crinkle patches remain red.

The most useful genetic marker is the mutation for white eyes. It has complete penetrance in the homozygote, no expression in the heterozygote, and is easily classified at all ages and in all environments. The other mutations fail to meet one or more of these criteria. For this reason and also because of complex interactions between the mutations affecting the eyes, linkage tests are tedious to carry out. At the present time, the only linkage relationship which has been established is that between the white locus and the sex locus (Bowen, 1965).

D. *Epistasis and tests for allelism* (*eye color genes:* r, g *and* w)

In order to determine if the three eye color mutations were allelic, the following stocks were crossed: garnet × red, garnet × white, and red × white. Because the F_1 progeny from the three crosses were wild-type, we conclude that the three mutations are not alleles.

The gene *w*, when homozygous, is epistatic to the gene *r*. Stock #10 breeds true for white-eyed males (X_wX_w; *r*/*r*) and red-eyed females (X_wY_+; *r*/*r*) because crossing over between the X and Y is suppressed in this stock.

White is also epistatic to garnet. Evidence for this is seen in the results of a cross of garnet-eyed shrimps: *g*/*g*; X_+Y_w females to *g*/*g*; X_+X_w males. Twenty-seven per cent (57/211) of the progeny were white-eyed. Of the 57 white-eyed shrimps, 53 were females and 4 were males resulting from crossing over.

E. *Discussion of the mechanism of gene action* (r, g *and* w)

The black eye pigment of wild-type *Artemia* is an ommochrome (Becker, 1942). Probably the gene for garnet eyes affects ommochrome degradation rather than ommochrome synthesis for two reasons: (1) retinular cells of young *g/g* shrimps contain normal black pigment, and (2) retinular cells of sexually mature *g/g* shrimps degenerate as a result of the action of the garnet gene.

We will discuss three alternative hypotheses for the mode of action of the gene for white eye color:

1. The gene *w* may act on the stroma of the eye pigment granule, either by causing a complete absence of the stroma or by producing a defect in its structure. This hypothesis would account for the fact that *w/w*; *r/r* and *w/w*; *g/g* shrimps have white eye color. The white eyes should be examined with the electron microscope to determine if pigment granules are absent or changed in structure. Nolte (1961) has reported a great reduction in the number of granules in the retinulae of *st/st* and *v/v Drosophila* which lack ommochrome pigment.

2. The gene *w* may alter retinular cell membrane permeability in such a way as to prevent the entrance of ommochrome precursors into the cell. This hypothesis would also account for the fact that the gene for white is epistatic to the genes for red and garnet.

3. (a) The genes for white and for red eyes may be changes in structural genes which code enzymes in the biosynthetic pathway of ommochrome eye pigment. Because white is epistatic to red, the enzyme controlled by the white locus would act earlier in the ommochrome synthetic pathway than the enzyme controlled by the red locus. (b) Along the same line of reasoning, it is possible that *w* and *r* may be changes at operator or repressor loci which indirectly control enzymes in ommochrome synthesis. Some doubts about hypothesis #3 have been raised by the discovery of mosaic shrimps with compound eyes which contain black and white retinular cells lying next to one another (described under section G of this paper). If the white cells lacked an ommochrome precursor, one might expect it to enter the white cells by diffusion from adjacent normally pigmented cells, resulting in a gradient of white, intermediate, and normal cells. Because no intermediate cells were seen, we conclude that if hypothesis #3 is valid, the gene w^+ must govern an enzyme which catalyzes the synthesis of a non-diffusable precursor of the ommochrome eye pigment.

E. *Modifications of unknown origin*

In a study of the effects of x-irradiation upon encysted blastulae (Bowen, 1963b) variants were discovered among the inbred descendants of shrimps in the 2-kr and 10-kr x-irradiation treatments. There were five independent occurrences of absence of setae on the exopodites and three occurrences of swollen abdomen. Other variants were: bent abdomen, kidney-shaped eyes, swollen branchiae, and a ventral median projection on the fifth segment of the abdomen. The only viable pure-breeding stock that could be developed from the progeny of x-irradiated shrimps was the garnet-eye stock.

Many variants occurred in non-irradiated stocks. One male had no antennae

whatsoever. Several animals in one *r/r* line showed deposition of the red pigment delayed until sexual maturity. Many shrimps in a wild-type stock lacked a median eye. There were five independent occurrences of shortened, twisted abdomens (in addition to stump, described above). These morphological variants could not be developed into mutant stocks for one of these reasons: the shrimps died without progeny, sib matings gave all wild-type F_2 progeny, the traits had low viability or penetrance, or the stock lost vigor with inbreeding.

F. *Mosaics*

Eighteen mosaics are described in Tables III and IV. Eleven were sex mosaics, three were mosaics combining genitalia and phyllopodia tissue, one was a female with abnormal legs on one side, one was a male lacking an antenna, and two were metanauplii which had eyes of unequal sizes. The last two hatched from cysts given a lethal dose of 50,000 r x-irradiation and died before maturity (Bowen, 1963b).

The three phyllopodia-genitalia mosaics (mosaics 1, 2 and 3 in Table III) suggested that structures within the phyllopodia are homologous with those in the genital segments. Mosaic #3 is a white-eyed male descended from a cross between a black-eyed mother (X_wY_+) and a white-eyed father (X_wX_w) from stock #9. On each side of the body, there is a normal testis filled with sperm and a male antenna. On the right side, the last four thoracic appendages are shortened and deformed. On the left side of the genital segments, there is a normal penis containing a vas and seminal vesicle. On the right, the external genitalia are a mixture of ovisac and penis structures proximally; distally, the structure becomes a phyllopodium (Fig. 21).

Of the 11 sex mosaics (numbers 8 to 18, in Tables III and IV), three were perfect bilateral gynandromorphs. Each had a testis containing sperm on one side and an ovary producing yolky eggs on the other. One has been described in detail earlier (Bowen and Hanson, 1962). Another sex mosaic (number 11 in Table III) consisted of male tissue except for the presence of shell glands filled with brown secretion. Mosaic No. 18 (Table IV) consisted of female tissue except for one perfect male antenna. The presence of a small amount of tissue characteristic of one sex in an animal composed for the most part of cells of the other sex is usually interpreted as evidence that sex is determined autonomously. We can be certain that if a sex hormone is present in *Artemia,* it does not suppress the differentiation of cells with the chromosome constitution of the opposite sex. In each of the 11 sex mosaics, the internal organs were male or female rather than intersexual in character. The external shape of the antennae and genitalia were sometimes intermediate, but this could be attributed to mixtures of cells with male or female genotypes in the epidermis. Therefore, all the mosaics were in accord with the hypothesis that each cell is either male or female in phenotype.

Although we know that female *Artemia* are XY and males are XX, we do not know whether the female phenotype is due to (1) female-determining gene(s) on the Y or to (2) the balance of the two sets of autosomes (bearing genes for femaleness) to the single X chromosome (bearing genes for maleness). Each of the five sex mosaics in Table IV resulted from the cross of a white male from stock #9 to a w/+ female. It was hoped that some insight into sex determination

TABLE III

Descriptions of thirteen mosaic shrimps

Mosaic no.	Code*	Description
1	s	Normal male antennae and male reproductive organs in genital segments. Five appendages on left side of thorax are mixtures of male and female genitalia.
2	s	Both testes filled with sperm, normal male antennae. External genitalia on both sides are a mixture of penis, ovisac, and phyllopod structures. Vas is continuous with internal structures of phyllopod.
3	s	On both sides, there is a normal male antenna and a testis filled with sperm. *Left side:* normal penis containing vas and seminal vesicle. *Right side:* the genitalia are a mixture of ovisac and penis structures proximally; distally there is a phyllopodium (Fig. 21).
4	s	Female with normal antennae, ovaries, and genitalia. On two thoracic segments, one leg is shortened, another slants dorsally.
5	px	Normal male genitalia and testis on both sides. One antenna has normal male shape; other is a broad stump.
6, 7	x	Two metanauplii; each has eyes of disparate size.
8, 9, 10	px	Three perfect bilateral gynandromorphs.
11	px	*Right side:* male antenna, testis filled with sperm, male genitalia (although penis lacks external spine). *Left side:* antenna is intermediate in shape and lacks frontal knob. Testis is filled with sperm. External genitalia are mixture of ovisac and penis structures; within lie seminal vesicle, vas, and shell glands (attached to vas deferens).
12	px	*Right side:* small lump in place of antenna, testis filled with sperm. Although penis is normal, seminal vesicle is straight and vas is missing. *Left side:* normal male antenna, testis filled with sperm; external genitalia are a mixture of ovisac and penis structures. A short spherical seminal vesicle is filled with sperm.
13	s	Perfect female antenna on left, intermediate antenna on right lacks frontal knob. Normal ovisac. In this immature specimen, the sex of the gonad and accessory organs could not be determined.

* x, mosaic hatched from x-irradiated cyst (50,000 r);
px, mosaic found in progeny of x-irradiated shrimps (2,000 or 10,000 r);
s, spontaneous occurence in non-irradiated stocks.

could be gained from study of these mosaics whose sex chromosomes carry eye color markers. However, no conclusions can be drawn because each of the mosaics in Table IV could have resulted from (1) a normal zygote with somatic non-disjunction occurring in an early cleavage division, (2) a binuclear oocyte with both nuclei fertilized, or (3) a binuclear oocyte with only one nucleus fertilized. Goldschmidt (1952, p. 123) reported finding binuclear oocytes in California *Artemia*. This problem is further complicated by crossing over between the X and Y; an estimate of crossover frequency in the five mothers can be obtained from the third vertical column in Table IV.

Histological examination of mottled eyes (in mosaics #17 and 18) revealed that retinular cells containing white pigment were adjacent to retinular cells with wild-type black pigment. In mosaic #14, white ommatidia were adjacent to red ommatidia which turned black as the animal aged. There were no intermediate cells bearing a reduced amount of dark pigment (see Fig. 15). This indicates that there is no diffusable substance produced by wild-type or by *r/r* retinulae which is lacking in white retinulae; that is, the type of pigment is determined

TABLE IV

Five mosaic progeny of non-irradiated parents
(w/w *fathers and* w/+ *mothers*)

Mosaic no.	Parents*		Siblings and progeny of similar matings	Description of mosaic	
	Mother	Father		Left side	Right side
14	$X_w^9 Y_+^5$	$X_w^9 X_w^9$	1 pigm. ♂	Red median eye	
			2 white ♀ ♀ 513 pigm. ♀ ♀ 379 white ♂ ♂	Some white retinular cells surrounded by *r/r* cells (red, changing to black at maturity). Normal male antenna.	White compound eye. Proximal segment of antenna male; distal part female.
				Male gonad, genitalia, and sperm present on both sides.	
15	$X_w^9 Y_+^9$	$X_w^9 X_w^9$	623 white ♂ ♂	Red median eye	
			877 pigm. ♀ ♀	Red compound eyes	
				Normal male antenna. Shell glands present. Mixed male and female genitalia.	Normal female antenna. Female ovisac and shell glands.
16	$X_w^9 Y_+^Q$	$X_w^9 X_w^9$	69 pigm. ♀ ♀ 59 white ♂ ♂ 8 white ♀ ♀ 15 pigm. ♂ ♂	White compound eye. Larger antenna, mixed male and female characteristics. Female gonad, yolky eggs. Genitalia mixed.	Black compound eye. Smaller antenna, mixed male and female characteristics. Female gonad, yolky eggs. Female genitalia; oviduct, 2 shell glands, half-uterus.
17	$X_+^{SF} Y_w^{11}$	$X_w^9 X_w^9$	264 white ♀ ♀ 294 pigm. ♂ ♂	Black and white retinular cells in eye.	White compound eye.
			31 white ♂ ♂ 36 white ♀ ♀	Normal female antennae, gonads and genitalia on both sides of body.	
18	$X_w^9 Y_+^{SF}$	$X_w^9 X_w^9$	149 white ♂ ♂	White and black (mottled) median eye	
			149 pigm. ♀ ♀ 8 white ♀ ♀	Black compound eye. Female antenna.	White compound eye. Male antenna.
			6 pigm. ♂ ♂	Male gonad, genitalia and sperm present on both sides.	

* In each genotype, subscripts indicate the allele on the sex chromosome; superscripts designate origin of the differential segment of the sex chromosome (inbred stocks 5, 9, 11; and wild populations: SF, San Francisco Bay; Q, Quemado, New Mexico).

autonomously by the genes rather than through the mediation of hormones or other diffusable substances.

Summary

1. Seven mutant genes of the brine shrimp have been studied. The mutation *s,* stump, shortens the abdomen; in extreme cases, all six abdominal segments are missing. An autosomal sex-limited mutation, *Cu,* curved, determines that females will have small curved antennae similar to those of the male. Two mutations (*w,* white and *r,* red) affect color of the eye and two mutations alter eye structure (*cy,* cyclops and *c,* crinkle). The garnet mutation, *g,* affects both color and structure of the eye.

2. Five of the mutant genes are autosomal, one (white) is partially sex-linked, and the mode of inheritance of one (cyclops) is not completely known.

3. Injections of India ink were used to demonstrate the distribution of phagocytic cells. These cells also take up pigment released by degenerating retinular cells in garnet-eyed shrimps.

4. The 11 sex mosaics are consistent with the hypothesis that each cell is male or female rather than intersexual in character.

5. Four shrimps had eyes which were mosaic for red and white or for black and white retinular cells. This suggests that eye pigment is determined autonomously; that is, there is no diffusable factor produced by red or wild-type retinular cells which is lacking in white cells.

6. The gene for white eyes, when homozygous, is epistatic to the genes for garnet and for red eyes. Three possible modes of action of the gene *w* are discussed.

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